

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A01N 47/40, C07C 331/04, 331/12	A1	(11) International Publication Number: WO 94/19948 (43) International Publication Date: 15 September 1994 (15.09.94)
(21) International Application Number: PCT/US94/02453 (22) International Filing Date: 11 March 1994 (11.03.94) (30) Priority Data: 030,610 12 March 1993 (12.03.93) US (71)(72) Applicants and Inventors: CHO, Cheon-Gyu [KR/US]; 110 West 39th Street, Baltimore, MD 21210 (US). POSNER, Gary, H. [US/US]; 3216 Timberfield Lane, Baltimore, MD 21208 (US). TALALAY, Paul [US/US]; 5512 Box-hill Lane, Baltimore, MD 21212 (US). ZHANG, Yuesheng [CN/US]; 105 Est 39th Street, Baltimore, MD 21210 (US). (74) Agents: HOSCHEIT, Dale, H. et al.; Banner, Birch, McKie & Beckett, Suite 1100, 1001 G Street, N.W., Washington, DC 20001-4597 (US).		(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: CHEMOPROTECTIVE ISOTHIOCYANATES (57) Abstract <p>Sulforaphane has been isolated and identified as a major and very potent phase II enzyme inducer in broccoli (<i>Brassica oleracea italica</i>). Sulforaphane is a monofunctional inducer, inducing phase II enzymes selectively without the induction of aryl hydrocarbon receptor-dependent cytochromes P-450 (phase I enzymes). Analogues differing in the oxidation state of sulfur and the number of methylene groups were synthesized, and their inducer potencies were measured. Sulforaphane is the most potent of these analogues. Other analogues having different substituent groups in place of the methylsulfinyl group of sulforaphane were also synthesized and assessed. Of these, the most potent are 6-isothiocyanato-2-hexanone and <i>exo</i>-2-acetyl-6-isothiocyanatonorbomane.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

CHEMOPROTECTIVE ISOTHIOCYANATES

This invention was made with support from the National Institutes of Health, Grant No. CA44530. The U.S. government therefore retains certain rights in the invention.

TECHNICAL FIELD OF THE INVENTION

This invention relates to compounds which stimulate mammalian enzymes which are involved in detoxication of carcinogens. More specifically, it relates to compounds which induce the activity of quinone reductase¹, glutathione transferases and other phase II enzymes, without inducing the activity of cytochromes P-450.

BACKGROUND OF THE INVENTION

Extrinsic factors, including personal life-styles, play a major role in the development of most human malignancies (Wynder, et al., *J. Natl. Cancer Inst.* 58:825-832 (1977); Higginson, et al., *J. Natl. Cancer Inst.* 63:1291-1298 (1979); Doll, et al., *J. Natl. Cancer Inst.* 63:1191-1308 (1981)). Cigarette smoking and

¹Abbreviation: QR, quinone reductase [NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2].

consumption of alcohol, exposure to synthetic and naturally occurring carcinogens, radiation, drugs, infectious agents, and reproductive and behavioral practices are now widely recognized as important contributors to the etiology of cancer. But perhaps most surprising is the inference that normal human diets play causative roles in more than one-third (and possibly even two-thirds) of human neoplasia (Wynder, et al., *J. Natl. Cancer Inst.* 58:825-832 (1977); Higginson, et al., *J. Natl. Cancer Inst.* 63:1291-1298 (1979); Doll, et al., *J. Natl. Cancer Inst.* 63:1191-1308 (1981)). Our food contains not only numerous mutagens and carcinogens but also a variety of chemicals that block carcinogenesis in animal models (Ames, *Science* 221:1256-1264 (1983); Ames, et al. *Proc. Natl. Acad. Sci. USA* 87:7777-7781 (1990); Ames, et al., *Proc. Natl. Acad. Sci. USA* 87:7782-7786 (1990); Carr, B.I., *Cancer* 55:218-224 (1985); Fiala, et al., *Annu. Rev. Nutr.* 5:295-321 (1985); Wattenberg, *Cancer Res. Suppl.* 43:2448s-2453s (1983); Wattenberg, *Cancer Res.* 45:1-8 (1985); Wattenberg, et al., *Diet, Nutrition and Cancer*:193-203 (1986)). Furthermore, carcinogens can even protect against their own toxic and neoplastic effects or those of other carcinogens -- i.e., carcinogens may act as anticarcinogens (Richardson, et al., *Cancer Res.* 11:274 (1951); Huggins, et al., *J. Exp. Med.* 119:923-942 (1964); Huggins, et al., *J. Exp. Med.* 119:943-954 (1964)).

Clearly, dietary modifications modulate cancer risk in various ways: for instance, through changes in caloric intake, by altering the consumption of nutritive and nonnutritive major components, and by providing exposure to

numerous minor chemicals that may be genotoxic or protective (Ames, *Science* 221:1256-1264 (1983); Ames, et al., *Proc. Natl. Acad. Sci. USA* 87:7777-7781 (1990); Ames, et al., *Proc. Natl. Acad. Sci. USA* 87:7782-7786 (1990); Carr, *Cancer* 55:218-224 (1985); Wattenberg, *Cancer Res. Suppl.* 43:2448s-2453s (1983); Wattenberg, L.W., *Cancer Res.* 45:1-8 (1985); Wattenberg, et al., *Diet, Nutrition and Cancer*:193-203 (1986); Tannenbaum, et al., *Adv. Cancer Res.* 1:451-501 (1953); National Research Council, *Diet, Nutrition and Cancer*, (1982); National Research Council, *Diet and Health: Implications for Reducing Chronic Disease Risk*, (1989); Creasey, *Diet and Cancer*, (1985); Knudsen, *Generic Toxicology of the Diet*, (1986)). Rational recommendations for modifying human diets to reduce the risk of cancer require identification of dietary carcinogens and chemoprotectors, even though interactions among such factors in modulating cancer development are complex (Patterson, et al. *Am. J. Public Health* 78:282-286 (1988)). Whereas extensive efforts have been made to identify dietary carcinogens and mutagens (Ames, *Science* 221:1256-1264 (1983); Ames, et al. *Proc. Natl. Acad. Sci. USA* 87:7777-7781 (1990); Ames, et al., *Proc. Natl. Acad. Sci. USA* 87:7782-7786 (1990)), chemoprotective components have received far less attention.

Numerous epidemiological studies suggest that high consumption of yellow and green vegetables, especially those of the family Cruciferae (mustards) and the genus *Brassica* (cauliflower, cress, brussels sprouts, cabbage, broccoli), reduces the risk of developing cancer of various organs (Graham, et al., *J. Natl. Cancer*

Inst. 61:709-714 (1978); Graham, *Cancer Res. Suppl.* 43:2448s-2453s (1983); Colditz, et al., *Am. J. Clin. Nutr.* 41:32-36 (1985); Kune, et al., *Nutr. Cancer* 9:21-42 (1987); La Vecchia, et al., *J. Natl. Cancer Inst.* 79:663-669 (1987); Le Marchand, et al., *J. Natl. Cancer Inst.* 81:1158-1164 (1989); You, et al., *J. Natl. Cancer Inst.* 81:162-164 (1989)). Moreover, administration of vegetables or of some of their chemical components to rodents also protects against chemical carcinogenesis (Wattenberg, *Cancer Res. Suppl.* 43:2448s-2453s (1983); Wattenberg, *Cancer Res.* 45:1-8 (1985); Wattenberg, et al., *Diet, Nutrition and Cancer* 193-203 (1986); Boyd, et al., *Food Chem. Toxicol.* 20:47-52 (1982)).

Well-documented evidence established that feeding of certain vegetables (e.g., brussels sprouts and cabbage) induces both phase I and phase II enzymes² in animal tissues (Conney, et al., *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 36:1647-1652 (1977); Spornins, et al., *J. Natl. Cancer Inst.* 66:769-771 (1981); Spornins, et al., *J. Natl. Cancer Inst.* 68:493-496 (1982); Aspary, et al., *Food Chem. Toxicol.* 21:133-142 (1983); Bradfield, et al., *Food Chem. Toxicol.* 23:899-904

²Enzymes of xenobiotic metabolism belong to two families (i) phase I enzymes (cytochromes P-450), which functionalize compounds, usually by oxidation or reduction; although their primary role is to detoxify xenobiotics, several cytochromes P-450 can activate procarcinogens to highly reactive ultimate carcinogens (Miller, et al., *Bioactivation of Foreign Compounds*, 3-28 (1985)); and (ii) phase II enzymes, which conjugate functionalized products with endogenous ligands (e.g., glutathione, glucuronic acid, sulfate) and serve primarily a detoxification role (Jakoby, et al., *J. Biol. Chem.* 265:20715-20718 (1990)). Quinone reductase (QR) is considered a phase II enzyme because it has protective functions (Prochaska, et al., *Oxidative Stress: Oxidants and Antioxidants*, 195-211 (1991)) is induced coordinately with other phase II enzymes, and is regulated by enhancer elements similar to those that control glutathione transferase (Favreau, et al., *J. Biol. Chem.* 266:4556-4561 (1991)).

(1985); Salbe, et al., *Food Chem. Toxicol.* 24:851-856 (1985); Whitty, et al., *Food Chem. Toxicol.* 25:581-587 (1987); Ansher, et al., *Hepatology* 3:932-935 (1983); Ansher, et al., *Food Chem. Toxicol.* 24:405-415 (1986)) and stimulates the metabolism of drugs in humans (Conney, et al., *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 36:1647-1652 (1977); Pantuck, et al., *Clin. Pharmacol. Ther.* 25:88-95 (1979); Pantuck, et al., *Clin. Pharmacol. Ther.* 35:161-169 (1984)). The elevations of enzymes that metabolize xenobiotics may be highly relevant to the protective effects of vegetables, since relatively modest dietary changes not only affected the metabolism of drugs (Ansher, et al., *Food Chem. Toxicol.* 24:405-415 (1986)) but also modified the ability of carcinogens to cause tumors in rodents (Tannenbaum, et al., *Adv. Cancer Res.* 1:451-501 (1953); National Research Council, *Diet, Nutrition and Cancer* (1982); National Research Council, *Diet and Health: Implications for Reducing Chronic Disease Risk* (1989); Creasey, *Diet and Cancer* (1985); Knudsen, *Genetic Toxicology of the Diet* (1986); Longnecker, et al., *Cancer* 47:1562-1572 (1981); Fullerton, et al., *Proc. Am. Assoc. Cancer Res.* 29:147 (1988); Li, et al., *Cancer Res.* 50:3991-3996 (1990)). There is now very good evidence that when phase II enzymes are induced, animals and cells are protected against the toxic and neoplastic effects of carcinogens. In fact, anticarcinogens have been identified based on their ability to induce phase II enzymes. (Reviewed in Talalay (1992) "Chemical protection against cancer by induction of electrophile detoxication (phase II) enzymes" in *Cellular and*

Molecular Targets of Chemoprevention, (V.L. Steele et al., eds.) CRC Press,
Raton, FL.)

There is a need in the art for the identification of specific compounds which are able to exert an anti-carcinogenic effect on mammals. Once identified, these chemoprotective compounds can be used as prophylactic medicaments or as food additives.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a pharmaceutical composition for cancer prevention.

It is another object of the invention to provide compounds which have cancer chemoprotection activity.

It is yet another object of the invention to provide a method for protecting against cancer development.

It is still another object of the invention to provide a food product which is supplemented with a chemoprotective compound.

These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment a pharmaceutical composition for cancer prevention is provided. The composition comprises an active ingredient which is sulforaphane ((-)-1-isothiocyano-(4R)-(methylsulfinyl)butane) (CAS 4478-93-7) or an analogue thereof, said analogue having a first moiety which is an isothiocyanate and a second moiety which is a polar functional group, wherein said analogue has a chain of one or more carbon

atoms linking said first and said second moieties, and wherein said analogue contains no pyridyl moieties.

In another embodiment of the invention compounds are provided which have cancer chemoprotection activity. The compounds include: 1-isothiocyanato-5-methylsulfonylpentane ($\text{CH}_3\text{-SO}_2\text{-(CH}_2)_5\text{-NCS}$) ((GHP 1003), 6-isothiocyanato-2-hexanone ($\text{CH}_3\text{CO(CH}_2)_4\text{NCS}$) (GHP 1105), *exo*-2-acetyl-6-isothiocyanatonorbornane (GHP 1066), *exo*-2-isothiocyanato-6-methylsulfonylnorbornane (GHP 1068), 6-isothiocyanato-2-hexanol (GHP 1106), 1-isothiocyanato-4-dimethylphosphonyl-butane (GHP 1078), *exo*-2-(1'-hydroxyethyl)-5-isothiocyanatonorbornane (GHP 1075), *exo*-2-acetyl-5-isothiocyanatonorbornane (GHP 1067), and *cis*- or *trans*-3-(methylsulfonyl)cyclohexylmethylisothiocyanate (GHP 1080 and 1079).

In yet another embodiment of the invention a method for protecting against cancer induction or progression is provided. The method comprises the step of: administering to a mammal a chemoprotective composition consisting essentially of sulforaphane ((-)-1-isothiocyanato-(4R)-(methylsulfinyl)butane) or an analogue thereof, said analogue having a first moiety which is an isothiocyanate functionality and a second moiety which is a polar functional group, wherein said analogue has a chain of one or more carbon atoms linking said first and said second moieties, and wherein said analogue contains no pyridyl moieties, in an amount effective in producing a cancer preventive effect.

In still another embodiment of the invention a food product is provided. The product has been supplemented with an active chemoprotective compound, wherein said compound is sulforaphane ((-)-1-isothiocyanato-(4R)-(methylsulfinyl)butane) or an analogue thereof, said analogue having a first moiety which is an isothiocyanate functionality and a second moiety which is a polar functional group, wherein said analogue has a chain of one or more carbon atoms linking said first and said second moieties.

These and other objects of the invention provide the public with positive means to lower the risk of developing cancers.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the structure of sulforaphane.

Figure 2 shows the fragmentation pattern of sulforaphane.

Figure 3 shows the synthesis of (GHP 1003).

Figure 4 shows the synthesis of (GHP 1066 and 1067).

Figure 5 shows the synthesis of (GHP 1068).

Figure 6 shows the synthesis of (GHP 1073).

Figure 7 shows the synthesis of (GHP 1075).

Figure 8 shows the synthesis of (GHP 1078).

Figure 9 shows the synthesis of (GHP 1079 and 1080).

Figure 10 shows the synthesis of (GHP 1105).

Figure 11 shows the synthesis of (GHP 1106).

DETAILED DESCRIPTION OF THE INVENTION

Chemoprotective activities have been detected in certain vegetables which are able to induce the activity of enzymes that detoxify carcinogens (phase II enzymes). One such activity has been detected in broccoli which induces quinone reductase activity and glutathione S-transferase activities in murine hepatoma cells and in the organs of mice. This activity has been purified from broccoli and identified as sulforaphane. Analogues of sulforaphane have been synthesized to determine structure-function relationships.

It is the discovery of the present invention that sulforaphane and its isothiocyanate analogues have chemoprotective activity in excess of previously discovered compounds. The analogues contain a moiety which is a polar functional group. This may be, for example, a sulfoxide, a ketone, a sulfone, a sulfide, a thioester, a thioether, a nitrile, a nitro, a carboxylic ester, a carboxylic acid, a halogen, a phosphine oxide, or a hydroxyl group. The isothiocyanate moiety and the polar functional group are linked by a chain of one or more carbon atoms. Preferably there are at least three carbon atoms in the chain. Typically there are three to five carbon atoms in the chain. The analogues do not contain pyridyl moieties.

The chemoprotective compounds of the present invention can be administered to mammals as a prophylactic against chemically induced cancers. The compounds can be formulated in suitable excipients for oral administration, for topical administration, or for parenteral administration. Such excipients are

well known in the art. According to the present invention, pharmaceutical compositions are those which are suitable for administration to humans or other mammals. Typically they are sterile, and contain no toxic, carcinogenic, or mutagenic compounds which would cause an adverse reaction when administered. Administration of the compounds can be performed before, during, or after exposure to the offending carcinogens or procarcinogens. Suitable doses to be administered are those which are sufficient to induce a demonstrable increase of phase II enzymes. This will typically not exceed 500 μ moles per kg per day, but may be much lower.

Sulforaphane and sulforaphene are known to be produced by plants, such as hoary cress, radish and other plants (Mislou, et al. (1965) *J. Am. Chem. Soc.* 87:665-666; Schmid, et al. (1948) *Helvet. Chim. Acta* 31:1017-1028; Hansen et al. (1974) *Acta Chem. Scand. Ser. B* 28:418-424). For the purposes of the present invention, they can be isolated from plants or synthesized. Bertoin, alyssin, erucin, erysolin, iberiverin, iberin, and cheirolin can also be isolated from plants; these compounds appear to be less active as inducers than sulforaphane and sulforaphene, at least in cell culture.

Other synthetic analogues of sulforaphane will preferably not be heteroaromatic and more preferably will not be aromatic. Such analogues include olefins, aliphatics, and non-aromatic ring compounds. Some examples of these are shown below in Table 3. The CD value provides a measure of the potency of the compounds as inducers of phase II enzymes, specifically quinone reductase. The

preferred compounds of the invention have CD values less than 1, although some established chemoprotectors have higher CD values.

Other analogues of sulforaphane can be used which are not specifically shown. The relative ability of the compound to induce the chemoprotective enzymes can be assessed as taught below, either by testing induction in cell lines, or in whole animals. The compounds can also be tested for the ability to suppress hepatoma formation in rats by 3-methylcholanthrene, 2-acetylaminofluorene, diethylnitrosamine, *m*-toluenediamine, and azo dyes. They can also be tested for the ability to block the neoplastic effects of diethylnitrosamine or benzo[*a*]pyrene on lung and forestomach of mice or of dimethylbenz[*a*]anthracene (DMBA) on mammary tumor formation in rats.

Also provided by the present invention are food products which have been supplemented with a chemoprotective compound of the present invention. The supplement may be isolated from plants or synthesized.

EXAMPLES

Example 1

This example describes the rapid cell culture assay which was used in the purification of a chemoprotective compound from broccoli.

Assay of Inducer Potency. Inducer activity was measured in Hepa 1c1c7 murine hepatoma cells grown in 96-well microtiter plates (Prochaska and Santamaria, *Anal. Biochem.* 169:328-336 (1988); Prochaska et al., *Proc. Natl. Acad. Sciences USA* 89:2394-2398 (1992)). QR activity (based on the formati n

of the blue-brown reduced tetrazolium dye) was measured with an optical microtiter plate scanner in cell lysates prepared in one plate, and the cell density was determined in the second plate by staining with crystal violet. Quantitative information on specific activity of QR, the inducer potency, and the cytotoxicity of the extract or compound tested is obtained by computer analysis of the absorbances. One unit of inducer activity is defined as the amount that when added to a single microtiter well doubled the QR specific activity. The CD value is the concentration of a compound required to double the quinone reductase specific activity in Hepa 1c1c7 murine hepatoma cells.

Sources of Vegetable and Preparation of Extracts. Vegetables were homogenized with 2 vol of cold water in a Waring Blendor at 4°C. The resultant soups were lyophilized to give dry powders, which were stored at -20°C. Portions (400 mg) of these powders were extracted for 6-24 hr with acetonitrile by shaking in glass vessels at 4°C. The extracts were filtered and evaporated to dryness. The residues were dissolved or suspended in acetonitrile or dimethyl formamide.

The specific activities of QR were raised nearly 6-fold at the highest extract concentrations tested, at which less than 20% cytotoxicity was observed. The inductions obtained with broccoli and with other vegetable extracts were proportional to the quantity of extract added over a reasonably wide range. The toxicities of these extracts were modest and were unrelated to their inducer potencies.

Extracts of a series of organically grown vegetables cultivated under a variety of conditions showed large differences in inducer potencies. Although many vegetable extracts induced QR, certain families were consistently more potent inducers. For example, where extracts of several Cruciferae had potent inducer activity, extracts of Solanaceae (peppers, potatoes, tomatoes) had low inducer activity. Of the 24 vegetables examined only 6 showed detectable toxicity; the others were nontoxic at the highest concentrations tested.

Cytotoxicity measurements are important because phase II enzyme inducers may be toxic and/or carcinogenic. Moreover, by use of mutant Hepa cells defective in aryl hydrocarbon receptor or cytochrome P-450 function (Zhang, et al., *Proc. Natl. Acad. Sci. USA* 89:2399-2403 (1992); Prochaska, et al., *Cancer Res.* 48:4776-4782 (1988); De Long, et al., *Carcinogenesis* 8:1549-1553 (1987)), our assay system can distinguish *monofunctional* inducers (which elevate phase II enzymes selectively), from *bifunctional* inducers (which elevate both phase I and II enzymes) (Prochaska, et al., *Cancer Res.* 48:4776-4782 (1988)). Such information is crucial for identification of chemoprotective enzyme inducers for potential use in humans. Ideally, such inducers should be monofunctional, because elevated activities of phase I enzymes may lead to carcinogen activation. The assay of phase II enzymes makes possible further detailed analysis of the effects of treatment of vegetables (e.g., breeding, mutagenesis, growth, storage, and cooking conditions) that might enhance or depress such induction.

This example describes the isolation of a potent major phase II enzyme inducer from broccoli.

Fractionation of acetonitrile extracts of SAGA broccoli by preparative reverse-phase HPLC with a water/methanol solvent gradient resulted in recovery of 70-90% of the applied inducer activity in the chromatographic fractions. Surprisingly, the majority (about 65-80% in several chromatographies) of the recovered activity was associated with a single and relatively sharp peak [fractions 18-23; eluted at 64-71% (vol/vol) methanol]. This HPLC procedure was therefore adopted as the first step of the larger-scale isolation of inducer activity.

Lyophilized SAGA broccoli was extracted three times with acetonitrile (35 ml/g) for 6 hr each at 4°C. The pooled extracts were filtered successively through 0.45- and 0.22- μ m porosity filters (discarding the insoluble material) and evaporated to dryness under reduced pressure on a rotating evaporator (<40°C). About 1 g of residue from 640 g of fresh broccoli (64 g of lyophilized powder) contained 3.6×10^6 units of inducer activity. The residue was mixed thoroughly with 120 ml of methanol/water (25/75, vol/vol) and the insoluble fraction was discarded. Although not all of the residue obtained from the extraction was soluble in aqueous methanol, the solvent partition procedure resulted in substantial purification without significant loss of inducer activity. Portions of the extract were dried in a vacuum centrifuge and dissolved in small volumes of dimethyl formamide (0.75-1.0 ml per 50 mg of residue), and 50-mg portions were subjected

to HPLC (nine runs). Fractions 18-23 from all runs were pooled, evaporated to dryness, applied in acetonitrile to five preparative silica TLC plates (100 x 200 x 0.25 mm), and developed with acetonitrile, which was run to the top of each plate three times. Four major fluorescence-quenching components were resolved, and nearly all (99%) of the inducer activity migrated at R_f 0.4. The active bands were eluted with acetonitrile, pooled, and fractionated by two runs on a second preparative reverse-phase HPLC in a water/acetonitrile gradient (20%-71%). Ultraviolet absorption and inducer activity were eluted in a sharp coincident peak (at 66% acetonitrile) that contained all of the activity applied to the column. Evaporation (<40°C) of the active fractions gave 8.9 mg of a slightly yellow liquid, which contained 558,000 inducer units (overall yield 15%) and migrated as a single band on TLC.

Example 3

This example describes the identification of the inducer isolated from broccoli, as described in Example 2.

The identify of the inducer was established by spectroscopic methods and confirmed by chemical synthesis. It is (-)-1-isothiocyanato-(4R)-(methylsulfinyl) butane, known as sulforaphane or sulphoraphane (CAS 4478-93-7). See Figure 1.

Sulforaphane has been synthesized (Schmid, et al., *Helv. Chim. Acta* 31:1497-1505 (1948)) and isolated from leaves of hoary cress (Procházka, *Collect. Czech. Chem. Commun.* 24:2429-2430 (1959)) and from other plants (Kjær, et al.,

Acta Chem. Scand. 12:833-838 (1958)), and the absolute configuration has been assigned (Mislow, et al., *J. Am. Chem. Soc.* 87:665-666 (1965)). The closely related olefin sulforaphene [4-isothiocyanato-(1R)-(methyl-sulfinyl)-1-(E)butene (CAS 2404-46-8)] has been isolated from radish seeds and other plants (Schmid, et al., *Helv. Chim. Acta* 31:1017-1028 (1948); Hansen, et al., *Acta Chem. Scand. Ser. B* 28:418-424 (1974)) and has also been synthesized (Cheung, et al., *J. Chem. Soc. Chem. Commun.*, 100-102 (1965); Balenović, et al., *Tetrahedron* 22:2139-2143 (1966)).

The following evidence establishes that (R)-sulforaphane is the inducer isolated from broccoli, UV spectrum (H_2O): λ_{max} 238 nm, ϵ_{238} 910 $M^{-1}cm^{-1}$; addition of NaOH (0.1 M) blue-shifted (λ_{max} 226 nm) and intensified (ϵ_{226} 15,300 $M^{-1}cm^{-1}$) this absorption band, consistent with the behavior of isothiocyanates (Svátek, et al., *Acta Chem. Scand.* 13:442-455 (1959)). IR (Fourier transform, neat): strong absorptions at 2179 and 2108 cm^{-1} and also at 1350 cm^{-1} , characteristic of isothiocyanates (Kjær, et al., *Acta Chem. Scand.* 9:1311-1316 (1955)). 1H NMR (400 MHz, C^2HCl_3): δ 3.60 (t, 2H, $J = 6.1$ Hz, $-CH_2-NCS$), 2.80-2.66 (m, 2H, $-CH_2-SO-$), 2.60 (s, 3H, CH_3-SO-), and 1.99-1.86 ppm (m, 4H, $-CH_2CH_2-$). ^{13}C NMR (400 MHz, C^2HCl_3): δ 53.5, 44.6, 38.7, 29.0, and 20.1 ppm. Mass spectrometry (fast atom bombardment; thioglycerol matrix) gave prominent peaks at 178 ($M + H$) $^+$ and 355 ($M_2 + H$) $^+$. Electron impact mass spectrometry gave a small molecular ion (M^+) at 177, and chemical ionization mass spectrometry gave a small molecular ion ($M + H$) $^+$ at 178 and

prominent fragment ions with masses of 160, 114, and 72, consistent with the fragmentation pattern shown in Figure 2. Precise masses of molecular and fragment ions obtained by electron impact mass spectrometry were 177.0286 (calculated for $C_6H_{11}NOS_2$, 177.0283), 160.0257 (calculated for $C_6H_{10}NS_2$, 160.0255), and 71.9909 (calculated for $C_2H_2NS_1$, 71.9908). In addition, for the mass 160 fragment, the peaks at 161 ($M + 1$) and 162 ($M + 2$) were 8.43% (calculated, 8.44%) and 9.45% (calculated, 10.2%), respectively, of the parent ion. Similarly, for the mass 72 fragment, the peaks at 73 ($M + 1$) and 74 ($M + 2$) were 3.42% (calculated, 3.32%) and 5.23% (calculated, 4.44%), respectively, of the parent ion. Hence the isotope compositions corrected for the natural isotope abundance (of ^{13}C , ^{15}N , ^{33}S , and ^{34}S) were consistent with the relative intensities of the $M + 1$ and $M + 2$ ions of both fragments. The optical rotation of the isolated material was $[\alpha]_D^{25} -63.6^\circ$ ($c = 0.5$, CH_2Cl_2), thus establishing that the product is largely, if not exclusively, the (—)-(R) enantiomer $[\alpha]_D -79^\circ$, -73.2° , -66° ; refs. 26, 30, and 38, respectively). The spectroscopic properties of synthetic (R,S)-sulforaphane were identical to those of the isolated product.

Example 4

This example describes the synthesis of sulforaphane (CAS 4478-93-7) and its closely related analogs, ibervin, erucin, berteroin, iberin, alyssin, cheirolin, erysolin, and 1-isothiocyanato-5-methylsulfonyl-pentane.

(R,S)-Sulforaphane (CAS 4478-93-7) was prepared according to Schmid and Karrer (Schmid, et al., *Helv. Chim. Acta* 31:1497-1505 (1948)) except that

gaseous trimethanol was replaced by sodium thionocarbonate. The sulfide analogues, $\text{CH}_3\text{—S—(CH}_2\text{)}_n\text{—NCS}$, where n is 4 [erucin (CAS 4430-36-8)] or 5 [berteroin (CAS 4430-42-6)] were prepared as described (Kjær, et al., *Acta Chem. Scand.* 9:1311-1316 (1955)), and the three-carbon analogue [iberverin (CAS 505-79-3)] was prepared from phthalimidopropyl bromide (Schmid, et al., *Helv. Chim. Acta* 31:1497-1505 (1948)). IR spectra of all three sulfide analogues showed strong absorptions near 2150 cm^{-1} , characteristic of isothiocyanates. ^1H NMR spectra of these compounds show sharp singlets at $\delta\ 2.10\text{ ppm}$ ($\text{CH}_3\text{—S}$ group). The sulfoxide analogues where n is 3 [iberin (CAS 505-44-2)] or 5 [alyssin (CAS 646-23-1)] were prepared by the same method as sulforaphane. IR spectra of these compounds showed strong absorptions near 2100 cm^{-1} , assigned to the —NCS group. ^1H NMR spectra also showed sharp singlets around $\delta\ 2.5\text{ ppm}$, consistent with the presence of the $\text{CH}_3\text{—SO}$ group. The sulfone analogues, $\text{CH}_3\text{—SO}_2\text{—(CH}_2\text{)}_n\text{—NCS}$, where n is 3 [cheirolin (CAS 505-34-0)], 4 [erysolin (CAS 504-84-7)], or 5 (unreported) were prepared by known methods (Schneider, et al., *Liebigs Ann. Chem.* 392:1-15 (1912)). ^1H NMR ($\delta\ \approx\ 2.9\text{ ppm}$, for $\text{CH}_3\text{—SO}_2\text{—}$) and IR spectra of these compounds were consistent with the structures. Every analogue within this example except 1-isothiocyanato-5-methylsulfonyl-pentane [$\text{CH}_3\text{—SO}_2\text{—(CH}_2\text{)}_5\text{—NCS}$] has been isolated from plants (Kjær, *Fortschr. Chem. Org. Naturst.* 18:122-176 (1960)).

Example 5

This example describes the inducer activity of the closely-related analogs of sulforaphane whose synthesis is described in the preceding Example.

Each of the analogs of sulforaphane was tested for the ability to induce QR in murine hepatoma cells by the assay described in Example 1. The following structure-function relationships were observed.

The chirality of the sulfoxide does not affect inducer potency, since isolated (R)-sulforaphane and synthetic (R,S)-sulforaphane gave closely similar CD values of 0.2-0.4 μ M. Sulforaphane is therefore the most potent monofunctional inducer that has been identified (Talalay (1989) *Adv. Enzyme Regul.* 28:237-250; Talalay et al., (1988) *Proc. Natl. Acad. Sci. USA* 85:8261-8265.³ Both (R)- and (R,S)-sulforaphane were relatively noncytotoxic: the concentrations required to depress cell growth to one-half were 18 μ M.

Sulforaphane and the corresponding sulfone (erysolin) were equipotent as inducers of QR, whereas the corresponding sulfide (erucin) was about one-third as active (Table 1). Oxidation of the side-chain sulfide to sulfoxide or sulfone enhanced inducer potency, and compounds with 4 or 5 methylene groups in the bridge linking $\text{CH}_3\text{S}-$ and $-\text{N}=\text{C}=\text{S}$ were more potent than those with 3 methylene groups.

³Benzylisothiocyanate has a reported CD value of 1.8 μ M; phenethylisothiocyanate has a CD of 2.0 μ M; ethylisothiocyanate has a CD of 30 μ M; propylisothiocyanate has a CD of 14 μ M; cyclohexylisothiocyanate has a CD of 14 μ M.

Table 1. Potency of induction of QR in Hepa 1c1c7 cells by sulforaphane and analogues

Compound	CD value, μ M		
	n = 3	n = 4	n = 5
$\text{CH}_3\text{---S---(CH}_2\text{)}_n\text{---N=C=S}$	3.5 (Ibervenni)	2.3 (Erucin)	1.7 (Berteroin)
$\text{CH}_3\text{---S---(CH}_2\text{)}_n\text{---N=C=S}$	2.4 (Ibenni)	0.4-0.8 (Sulforaphane)	0.95 (Alyssin)
$\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3\text{---S---(CH}_2\text{)}_n\text{---N=C=S} \\ \parallel \\ \text{O} \end{array}$	1.3 (Cheirolin)	0.82 (Erysolin)	0.98



Mutants of Hepa 1c1c7 cells defective in the Ah (aryl hydrocarbon) receptor or expression of cytochrome P-450IA1 can distinguish monofunctional inducers (which induce phase II enzymes selectively) from bifunctional inducers (which elevate both phase I and II enzymes) (De Long, et al., *Carcinogenesis* 8:1549-1553 (1987); Prochaska, et al., *Cancer Res* 48:4776-4782 (1988)). When sulforaphane was tested with the BP^ccl mutant (Miller, et al., *J. Biol. Chem.* 258:3523-3527 (1983)) (defective in transport of the liganded Ah receptor to the nucleus), and the cl mutant (Hankinson et al., (1985) *J. Biol. Chem.* 260:1790-1795) (which synthesizes inactive cytochrome P-450IA1), induction of QR was normal (data not shown). Sulforaphane is, therefore, like benzyl isothiocyanate, a monofunctional inducer (Prochaska, et al., *Cancer Res* 48:4776-4782 (1988)) and is unlikely to elevate activities of cytochromes P-450 that could activate carcinogens.

Example 6

This example demonstrates that the anti-cancer agents of the present invention are active in whole animals as inducers of phase II xenobiotic metabolism enzymes.

When synthetic (R,S)-sulforaphane, erysolin, and erucin were administered to female CD-1 mice by gavage (De Long, et al., *Cancer Res.* 45:546-551 (1985)), induction of QR and glutathione transferase activities was observed in the cytosols of several organs (Table 2). Sulforaphane and erucin (in daily doses of 15 μ mol for 5 days) raised both enzyme activities 1.6- to 3.1-fold in liver, forestomach, glandular stomach, and mucosa of proximal small intestine, and to a lesser degree in lung. The sulfone (erysolin) was more toxic, but even 5- μ mol daily doses for 5 days elevated the specific activities of these enzymes in some tissues examined. We therefore conclude that sulforaphane and its analogues not only induce QR in Hepa 1c1c7 murine hepatoma cells but also induce both QR and glutathione transferase activities in a number of murine organs.

Table 2. Induction of QR and glutathione S-transferase (GST) in mouse tissues by sulforaphane and analogues

Inducer	Dose, μmol per mouse per day	Enzyme	Liver	Ratio of specific activities (treated/contr 1)			
				Forestomach	Glandular stomach	Proximal small intestine	Lung
$\text{CH}_3\text{---S---}(\text{CH}_2)_4\text{---NCS}$ Erucin	15	QR GST	2.19 ± 0.06 1.86 ± 0.08	$1.64 \pm 0.18^*$ 2.51 ± 0.11	1.72 ± 0.11 2.07 ± 0.08	3.10 ± 0.20 3.00 ± 0.21	1.66 ± 0.13 1.41 ± 0.11
$\text{CH}_3\text{---S---}(\text{CH}_2)_4\text{---NCS}$  Sulforaphane	15	QR GST	2.45 ± 0.07 1.86 ± 0.08	$1.70 \pm 0.18^*$ 1.98 ± 0.08	2.35 ± 0.06 2.97 ± 0.08	2.34 ± 0.19 2.13 ± 0.20	1.37 ± 0.14 1.17 ± 0.09
$\text{CH}_3\text{---S---}(\text{CH}_2)_4\text{---NCS}$  Erysolin	5	QR GST	1.62 ± 0.09 $1.08 \pm 0.11^*$	$1.05 \pm 0.21^*$ $1.45 \pm 0.15^*$	$1.57 \pm 0.08^*$ $1.94 \pm 0.10^*$	$1.22 \pm 0.20^*$ $0.87 \pm 0.20^*$	1.00 ± 0.11 1.09 ± 0.13

The compounds were administered to 6-week-old female CD-1 mice 14 or 5 mice per group by gavage in indicated single daily doses in 0.1 ml of Emulphor EL 620P (GAF, Linden, NJ) for 5 days. Cytosols were prepared from the tissues 24 hr after the last treatment and assayed for enzyme activities (glutathione S-transferase was measured with 1-chloro-2,4-dinitrobenzene). The specific activities ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ \pm SEM) of organs of vehicle-treated control mice were as follows: Liver: QR, 47 ± 0.70 ; GST, 1014 ± 69 ; Forestomach: QR, 1038 ± 155 ; GST, 118 ± 74 ; Glandular stomach: QR, 3274 ± 85 ; GST, 1092 ± 81 ; Small intestine: QR, 644 ± 119 ; GST, 1372 ± 266 ; Lung: QR, 54 ± 5.8 ; GST, 43 ± 34 . Data are presented as mean \pm SEM. All ratios were significantly different from 1.0 with $P < 0.01$, except for * , $P < 0.05$, and $^+$, $P > 0.05$.

Example 7

This example describes the synthesis of *exo*-2-acetyl-6-isothiocyanatonorbomane (GHP 1066 and 1067).

From 1a (mixture)

To a 100 ml 3-neck round bottomed flask equipped with a magnetic stirring bar, dropping funnel and reflex condenser were placed 2.0 g (14.7 mmol) of 2-acetyl-5-norbomene (Aldrich Chemical Co.) and 10 ml of benzene. To this solution was added at room temperature (RT) a mixture of 2.1 g of conc. sulfuric acid and 1.0 ml of water slowly using a dropping funnel. After 4 days at RT, the reaction mixture was filtered through a sintered glass funnel. The filtered white solid was washed with 50 ml of ether. The combined organic solution was then washed with water and brine successively, dried over MgSO_4 , and concentrated in vacuo to afford a tan oil. Subsequent purification via flash silica-gel column chromatography (20/80, ether/hexane) afforded 1.73 g of product (60% yield, colorless oil) as a mixture of 4 stereoisomers based on ^1H NMR analysis (2a: 2b: 2c: 2d 36:39; 8:17). Purification by HPLC (silica-semi prep, 97/3 hexane/EtOAc, 10 ml/min) gave 2a (GHP 1066) in 22% overall yield.

From 1b (exo-only)

The same mixture as the above (1b:0.665 g) was stirred for 40 hr at 50°C. After the same work-up and column chromatography, 0.970 g of product (70% yield) was obtained as a mixture of 4 stereoisomers (2a: 2b: 2c: 2d 32:47; 1:20).

^1H NMR (400 MHz, CDCl_3) δ 3.64 (dd, $J = 7.6$, $J = 2.8$ Hz, 1H), 2.71(bs, 1H), 2.43(dd, $J = 4.5$ Hz, $J = 3.6$ Hz, 1H), 2.31(dd, $J = 8.4$ Hz, $J = 6.0$ Hz, 1H), 2.17(s, 3H), 1.83-1.67(m, 2H), 1.58-1.54 (m, 2H), 1.38-1.30(m, 2H); ^{13}C NMR (CDCl_3) δ 58.4, 50.9, 46.6, 40.0, 35.4, 33.6, 31.5, 28.8 (CO and NCS were not detected); FT-IR (CHCl_3 , cm^{-1}) 2955, 2132, 2085, 1708, 1343; HRMS cald. for $\text{C}_{10}\text{H}_{13}\text{NOS}$ 195.0719, found 195.0719.

Characterization of 2b (GHP 1067) ^1H NMR (400 Mhz, CDCl_3) δ 3.61 (dd, $J = 7.2$, $J = 2.8$ Hz, 1H), 2.58 (d, $J = 4.4$ Hz, 1H), 2.53 (d, $J = 4.5$ Hz, 1H), 2.35 (dd, $J = 8.5$ Hz, $J = 3.4$ Hz, 1H), 2.16 (s, 3H), 2.0 (dt, $J = 13.1$ Hz, 5.0, 1H), 1.91-1.86 (m, 1H), 1.79-1.75 (m, 1H), 1.55-1.52 (m, 1H), 1.37-1.33 (m, 1H), 1.26-1.22 (m, 1H); FT-IR (CHCl_3) 2978 cm^{-1} , 2179, 2146, 2085, 1708, 1449, 1343; Anal. cald. for $\text{C}_{10}\text{H}_{13}\text{NOS}$: C, 61.50; H, 6.71; N, 7.17; S, 16.42 found C, 61.64; H, 6.72; N, 7.12; S, 16.53.

Example 8

This example describes the synthesis of 1-isothiocyanato-5-methylsulfonylpentane (GHP 1003).

Preparation of 2

2 was prepared according to the literature procedure, and the spectral data match the literature values. Kjaer et al. *R. Acta. Chem. Scan.* 1955, 1311.

Preparation of 3

3 was prepared according to the literature procedure, and the spectral data match the literature values. Kjaer et al., *supra*.

Preparation of 4

2 was prepared according to the literature procedure, and the spectral data match the literature values.

Preparation of 5 (GHP 1003)

To a flask charged with 50 mg (0.3 mmol) of 4 and 0.8 ml of H₂O were added a solution of 0.03 ml of CSeCl₂ in 0.3 ml of CHCl₃ and 0.5 ml of 5% NaOH at RT. After 30 min, the reaction mixture was extracted with 20 ml of CH₂Cl₂ (10ml x 2). The combined organic solution was dried over MgSO₄, concentrated in vacuo, and purified by preparative TLC (6/4 EtOAc/hexane) to afford 16 mg (0.08 mmol) of 5 (GHP 1003, 26% from 3) as white solids. ¹H NMR (400 MHz, CDCl₃) δ 3.50 (t, J = 6.4 Hz, 2H), 2.98 (t, J = 4.1 Hz, 2H), 2.86 (s, 3H), 1.87-1.82 (m, 2H), 1.74-1.66 (m, 2H), 1.58-1.52 (m, 2H); FT-IR 3025 cm⁻¹, 2931, 2191, 2097, 1449, 1314, 1132.

Example 9

This example describes the synthesis of *exo*-2-isothiocyanato-6-methylsulfonylnorbornane (GHP 1068).

Preparation of 2a and 2b

The same procedure as described for GHP 1063 was used except that the reaction mixture was stirred for 6 days at 65°C. After work-up, 2a (17% yield)

were isolated by flash column chromatography (silica gel, 100% ether → 100% EtOAc). 2a (GHP 1064) was recrystallized from CH₂Cl₂/ether/hexane to afford ivy-leaf shaped crystals (mp; 142-143°C) in 12% yield. 2b (GHP 1068) was recrystallized from ether to afford small needles (mp; 82-82.5°C) in 4% yield.

Characterization of 2a

¹H NMR (400 MHz, CDCl₃) δ 3.66 (t, J=6.8 Hz, 1H), 2.90 (bs 1H), 2.86 (s, 3H), 2.80 (dd, J = 8.0 Hz, 2.8, 1H), 2.12 (td, J = 14.0 Hz, 5.2, 1H), 2.03 (dt, J = 12.0 Hz, 2.2 1H), 1.88-1.62 (m, 5H); FT-IR (CHCl₃) 3025 cm⁻¹, 2120, 2073, 1320; Anal. calcd. for C₉H₁₃NO₂S₂: C, 46.73; H, 5.66; N, 6.06; S, 27.72. found C, 46.74; H, 5.67; N, 6.11; S, 27.64.

Characterization of 2b

¹H NMR (400 MHz, CDCl₃) δ 3.65 (dd, J = 6.8 Hz, 2.8, 1H), 2.98 (bs, 1H), 2.87 (s, 3H), 2.76 (dd, J = 6.8, 1.2, 1H), 2.58 (bs, 1H), 2.06-1.61 (m, 6H); FT-IR (CHCl₃) 3025 cm⁻¹, 2978, 2191, 2120, 2085, 1349, 1308, 1138.

Example 10

This example describes the synthesis of *cis*-1-isothiocyanate-4-methylsulfonylcyclohexane (GHP 1073).

Preparation of 2

In an autoclave were placed 11.38 g (94.0 mmol, 10 eq.) of butadiene sulfone, 1.00 g (9.4 mmol) of 1, 0.2 g of hydroquinone as a polymerization inhibitor and 20 ml of absolute EtOH. Upon stirring for 15 min., the reaction mixture was sealed and heated at 110°C. After 60 hr., the reaction mixture was

cooled, and poured into 60 ml of 17% NaHCO_3 . After 10 min., the aqueous solution was extracted with ether (2 x 50 ml). The combined ether solution was dried over MgSO_4 , concentrated and chromatographed (60/40 ether/hexane) to afford 1.30 g (8.1 mmol, 85 % yield) of 2 as a brown oil. An aliquot was distilled under the reduced pressure to give a colorless liquid for analysis.

Preparation of 3

To a flask charged with 342 mg (0.63 mmol) of $\text{Hg}(\text{SCN})_2$ was added a premixed solution of I_2 in 8 ml of benzene. After 30 min. at room temperature (RT), to the mixture was added 202 mg (1.26 mmol) of 2 dissolved in 1 ml of benzene, and the flask containing the reaction mixture was wrapped with aluminum foil and stirred for 7.5 days at RT under argon atmosphere. The reaction mixture was then diluted with 20 ml of ether, washed with aqueous KI, $\text{Na}_2\text{S}_2\text{O}_3$, brine, dried over, and concentrated in vacuo. Flash column chromatography (1/1 ether/hexane) afforded 20 mg (0.06 mmol) of 3 (5% yield) as an oil along with 3 other isomers (11% yield).

Preparation of 4

To a flask charged with 21 mg (0.07 mmol) of 3 and 1 ml of benzene was added 0.05 ml (0.2 mmol, 3 eq.) of Bu_2SnH at RT. After 10 hr., the reaction mixture was treated with 35 mg of DBU (1,8-diazabicyclo[5,4,0]undec-7-ene) and 1 ml of wet ether. The resulting mixture was filtered off, concentrated and chromatographed (100% ether \rightarrow 100% EtOAc) to afford 7.3 mg (0.03 mmol) of 4 (GHP 1073, 55% yield) as a white solid (mp; 123°C).

¹H NMR (400 MHz, CDCl₃) δ 4.09 (t, J = 3.2 Hz, 1H), 2.86 (s, 3H), 2.87-2.80 (m, 1H), 2.23-2.20 (m, 4H), 1.96-1.85 (m, 2H), 1.71-1.59 (m, 2H); FT-IR (CHCl₃) 3025 cm⁻¹, 2943, 2085, 1302.

Example 11

This example describes the synthesis of *exo*-2-(1'-hydroxyethyl)-5-isothiocyanatonorbomane (GHP 1075).

To a flask charged with 37.3 mg (0.19 mmol) of 1 (GHP 1067) and 1.5 ml of MeOH was added 11.0 mg (0.29 mmol) of NaBH₄ at 0°C. After 20 min., the excess NaBH₄ was quenched with a few drops of H₂O, diluted with ether, dried over MgSO₄, and concentrated in vacuo. Preparative TLC (80/20 ether/hexane) afforded 21 mg (0.11 mmol) of 2 (GHP 1075, 56% yield) as a white solid (mixture of 2 diastereomers based on ¹H NMR).

¹H NMR (400 Mhz, CDCl₃) δ 3.58-3.54 (m), 3.49-3.45 (m), 3.39-3.32 (m), 2.54-2.46 (m), 2.18-2.16 (d), 1.80-1.65 (m), 1.55-1.20 (m), 1.19 (d, J = 6.0 Hz); FT-IR (CHCl₃) 3613 cm⁻¹, 2966, 2872, 2097, 1343.

Example 12

This example describes the synthesis of 1-isothiocyanato-4-dimethylphosphonyl-butane (GHP 1078).

Preparation of 2

To a 25 ml flame dried round bottomed flask charged with 15.2 ml (45.6 mmol) of MeMgCl (Aldrich Chemical Co., 3.0 M in THF) was added 1.5 ml

(11.41 mmol) of diethyl phosphite 1 while the internal temperature was maintained around 25°C with occasional cooling with ice-water bath. After 1 hr, the mixture was cannulated into the flask charged with 2.55 ml (22.82 mmol) of dibromobutane and 15 ml of THF at 0°C under Ar atmosphere. Upon addition, the reaction mixture was heated under reflux for 5 hr, cooled, and dumped into 30 ml of cold dil. HCl. The resulting aqueous solution was extracted with CHCl₃ (3x50 ml), and the organic solution was washed with sat. K₂CO₃, dried over K₂CO₃, and concentrated in vacuo to give 2.48 g of crude product as a tan oil. Purification by flash column chromatography (silica-gel, 8/2 EtOAc/MeOH→6/4 EtOAc/hexane) afforded 0.72 g (3.42 mmol) of 2 as a colorless oil.

Preparation of 3

In a 100 ml round bottomed flask were placed 0.733 g (3.44 mmol) of 2, 0.766 g of potassium phthalimide and 20 ml of DMF. The mixture was heated under reflux for 4 hr, cooled and dumped into 60 ml of CHCl₃. The organic solution was washed with H₂O dried over NaHCO₃, and concentrated in vacuo to afford 0.92 g of 3 as a white solid (used for next reaction without further purification).

Preparation of 4

To a flask charged with 0.1 g of 3 was added 4 ml of methanolic hydrazine (0.2 M) at RT. After 14 hr at RT, the reaction mixture was concentrated, and the residue was treated with 5 ml of 1 N HCl, washed with CHCl₃, strongly basified with solid NaOH. The basified solution was then extracted with CHCl₃ (2x20 ml),

and the combined organic solution was dried over K_2CO_3 , and concentrated in vacuo to 33 mg (0.22 mmol) of 4 as a white solid (used for next reaction without further purification).

Preparation of 5 (GHP 1078)

To a flask charged with 33 mg (0.22 mmol) of 4 and 1 ml of $CHCl_3$ were added at RT 0.02 ml (0.27 mmol) of $CSCL_2$ and 0.3 ml of 1 N NaOH. After 35 min at RT, the reaction mixture was partitioned between 10 ml $CHCl_3$ of and 10 ml H_2O . The separated organic layer was dried over $MgSO_4$, concentrated in vacuo and chromatographed (silica-gel, 8/2 EtOAc/MeOH) to afford 29 mg (0.15 mmol) of 5 (GHP 1078) as a reddish yellow oil. 1H NMR (400 MHz, $CDCl_3$) δ 3.54 (t, $J = 6.0$ Hz, 2H), 1.82-1.70 (m, 6H), 1.48 (s, 3H), 1.44 (s, 3H); FT-IR ($CHCl_3$) 2941 cm^{-1} , 2191, 2097, 1302, 1173; ^{13}C NMR (400 MHz $CDCl_3$) δ 44.5, 30.6 (d, $J = 20.2$ Hz, 1C), 30.7 (d, $J = 34.7$ Hz, 1C), 19.3, 16.2 (d, $J = 69$ Hz, 2C); ^{31}P NMR ($CDCl_3$) δ 46.1; HRMS cald. for $C_7H_{14}NOPS$ 191.0534, found 195.0536.

Example 13

This example describes the synthesis of *cis*- or *trans*-3-(methylsulfonyl)cyclohexylmethylisothiocyanate (GHP 1079 or 1080).

Preparation of 2

2 was prepared according to the literature procedure, and the spectral data matched the literature values. Kozikowski, A.; Ames, A. *Tetrahedron* 1985, 4821.

Preparation of 3

To a 100 ml round bottomed flask were placed 0.438 g (3.0 mmol) of 2, 1.418 g of 2,4,6-triisopropylbenzenesulfonohydrazide (prepared according to literature procedure; Jirieny, J.; Orere, D.; Reese, C. J. *Chem. Soc. Perkin Trans. I*, 1980, 1487) and 8 ml of MeOH at RT. After 1 hr, 0.739 g of KCN was added at RT, and the resulting mixture was heated under gentle reflux for 3 hr. The reaction mixture was cooled, diluted with 20 ml of H₂O, and extracted with CH₂Cl₂ (2x20 ml). The organic solution was subsequently washed with aq. NaHCO₃, dried over MgSO₄, concentrated in vacuo and purified by flash column chromatograph (8/2 hexane/EtOAc) to afford 0.360 g (2.3 mmol) of 3 (76 % yield) as a yellow oil.

Preparation of 4

To a flask charged with 0.36 g (2.32 mmol) of 3 and 10 ml of aqueous MeOH (9/1 v/v MeOH/H₂O) was added 2.75 g (4.64 mmol) of OXONE (2KHSO₅, KHSO₄, K₂SO₄) at RT. After 24 hr, the reaction mixture was filtered through a sintered glass funnel, and the filtered solid material was washed with 50 ml of CHCl₃. The combined organic solution was washed with H₂O, dried over MgSO₄, and concentrated in vacuo to afford 0.246 g (1.31 mmol) of 4 (57 % yield) as a colorless oil. This material was used in the next reaction without purification.

Preparation of 5

To a LiAlH₄ suspension in 10 ml of anhydrous ether was cannulated 0.246 g (1.31 mmol) of 4 dissolved in 3 ml of THF at RT. Upon addition, the reaction

mixture was heated under reflux. After 2.5 hr, the reaction mixture was cooled, quenched with 0.5 ml of H₂O and 0.5 ml of 5% of NaOH, and filtered through a sintered glass funnel. The solid material filtered was thoroughly washed with ether. The combined organic solution was dried over K₂CO₃ and concentrated in vacuo to afford 0.15 g (0.78 mmol) of 5 (60% yield) as a colorless oil. This material was used in the next reaction without purification.

Preparation of 6a (GHP 1079) and 6b (GHP 1080)

To a flask charged with 0.15 g (0.78 mmol) of 5 and 3 ml of CHCl₃ were added 0.07 ml of CCl₄ and 1.5 ml of 5% of NaOH at RT. After 1 hr, the reaction mixture was diluted with 10 ml of CH₂Cl₂, washed with H₂O and brine, dried over MgSO₄, concentrated in vacuo, and chromatographed (silica-gel, 1/1 hexane/EtOAc) to give 0.123 g (0.53 mmol) of products (67% yield) as a mixture 6a and 6b (1:1 ratio). HPLC (40/60 EtOAc/hexane) separation afforded GHP1079 and GHP 1080 (both as colorless oil). 6a (GHP 1079): ¹H NMR (400 MHz, CDCl₃) δ3.50 (d, J = 6.8 Hz, 2H), 3.09-3.03 (m, 1H), 2.88 (s, 3H), 2.45-2.37 (m, 1H), 2.14-2.07 (m, 1H), 1.98-1.84 (m, 4H), 1.74-1.66 (m, 1H), 1.59-1.41 (m, 2H); FT-IR (CHCl₃) 3013 cm⁻¹, 2943, 2872, 2191, 2097, 1449, 1308; ¹³C NMR (CDCl₃) δ52.6, 43.5, 33.6, 28.0, 22.5, 22.1, 19.5, 14.9; HRMS calc. 233.0544 found 233.0545. 6b (GHP 1080): ¹H NMR (400 MHz, CDCl₃) δ3.47 (d, J = 6.0 Hz, 2H), 2.92-2.82 (m, 1H), 2.84 (s, 3H), 2.28-2.20 (m, 2H), 2.04 (tt, J = 6.8 Hz, 3.0, 1H), 1.87-1.75 (m, 2H), 1.53-1.27 (m, 3H), 1.06 (tq, J = 12.2 Hz, 3.6, 1H); FT-IR (CHCl₃) 3025 cm⁻¹, 2931, 2861, 2191, 2097, 1449,

1308; ^{13}C NMR (CDCl_3) δ 56.5, 45.5, 32.6, 32.5, 23.9, 23.8, 20.0, 19.1; HRMS calc. 233.0544 found 233.0548.

Example 14

This example describes the synthesis of 6-isothiocyanato-2-hexanone ($\text{CH}_3\text{CO}(\text{CH}_2)_4\text{NCS}$)(GHP 1105).

Preparation of 2

To a flask charged with 2.252 g (19.22 mmol) of 1 and 20 ml of chloroform were added 40 ml of TMSCl . The mixture was heated around 45°C , then cooled to room temperature (RT). To this mixture was added 1.21 ml of CS_2 at RT, and the resulting solution was cooled to 0°C and treated with 8 ml of Et_3N . After 10 min, the reaction mixture was warmed to RT, and stirred for 2 hr, then cooled to 0°C , and treated with 2.0 ml of methyl chloroformate. After 45 min at 0°C , the reaction mixture was warmed at RT, diluted 75 ml of hexane, filtered off, and concentrated in vacuo. The residue was dissolved in 75 ml of THF at 0°C , then 7 ml of H_2O was added to it. After 1.5 days, the reaction was dried over MgSO_4 , concentrated and chromatographed (silica-gel, 20/80 EtOAc/hexane \rightarrow 1/1 EtOAc/hexane) to afford 1.563 g (9.80 mmol) of 2.

Preparation of 3 (GHP 1105)

A mixture of 0.3 g (1.89 mmol) of 2, 0.18 ml of SOCl_2 and 10 ml of CHCl_3 was heated under reflux for 2 hr. Upon removal of solvent, the residue was redissolved with 2 ml of dry ether. To this solution was added Me_2CuLi (prepared from CuI and 2MeLi in ether) at -78°C . After 2 hr at -78°C , the

reaction mixture was quenched with sat. NH_4Cl , warmed to RT, and extracted with ether. The organic solution was dried over MgSO_4 , concentrated in vacuo, and chromatographed (silica-gel, 30/70 ether/hexane) to afford 0.022 g (0.13 mmol) of 3 (7% yield from 2) as an oil. ^1H NMR (300 MHz, CDCl_3) δ 3.55-3.49 (m, 2H), 2.53-2.47 (m, 2H), 2.16 (s, 3H), 1.72-1.68 (m, 4H); FT-IR (CHCl_3) 3019 cm^{-1} , 2191, 2112, 1715, 1224.

Example 15

This example describes the synthesis of 6-isothiocyanato-2-hexanol (GHP 1106).


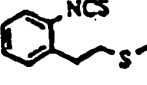

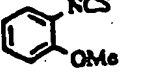

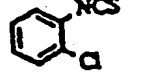

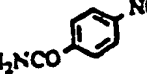


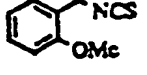

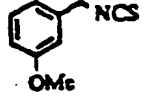


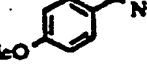

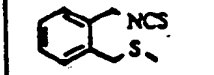
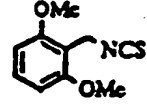
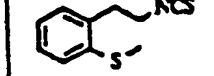
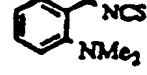
To a flask charged with 15.0 mg (0.1 mmol) of 1 and 2 ml of EtOH was added 3.6 mg (0.1 mmol) of NaBH_4 at 0°C . After 10 min, the reaction mixture was treated with 20 drops of H_2O , dried over MgSO_4 , concentrated in vacuo, and chromatographed (30/70 ether/hexane) to afford 7.5 mg (0.05 mmol) of 2 (GHP 1106, 49% yield) as a liquid.

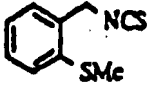

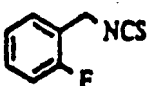

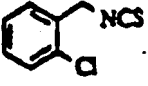

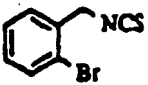

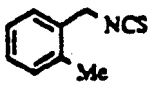
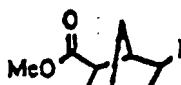
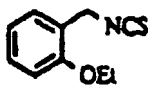

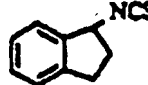

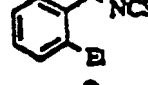
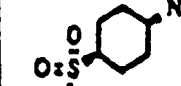
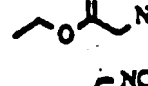
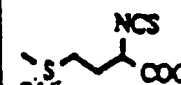




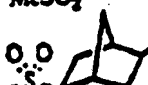
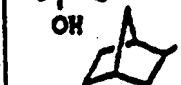

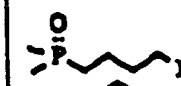
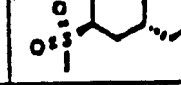
^1H NMR (400 MHz, CDCl_3) δ 3.87-3.77 (m, 1H), 3.53 (t, $J = 6.6$ Hz, 2H), 1.78-1.68 (m, 2H), 1.50-1.35 (m, 4H), 1.21 (d, 6.2 Hz, 3H); FT-IR (CHCl_3) 3615 cm^{-1} , 2945, 2180, 2107, 1375.

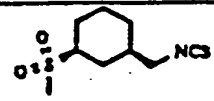


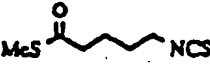




Example 16

This example describes the relative inducer activity of a large variety of sulforaphane analogs.

Analogues were synthesized and tested in the hepatoma cell assay described in Example 1. The results are shown in Table 3.

Table 3					
	STRUCTURE	CD (μ M)		STRUCTURE	CD (μ M)
GHP 1001		1.71	GHP 1023		100
GHP 1002		0.94	GHP 1031		100
GHP 1003		0.98	GHP 1032		100
GHP 1004		0.83	GHP 1033		100
GHP 1005	 sulforaphane	0.20			
GHP 1006		2.30	GHP 1041		2.41
GHP 1007		0.82	GHP 1042		8.65
GHP 1008		3.52			
GHP 1009		2.36	GHP 1043		25
GHP 1010		1.32			
GHP 1021		4.3	GHP 1044		5.8
GHP 1022		7.4			
			GHP 1045		12.5

GHP 1046		6.8	GHP 1066		0.27
GHP 1047		13.1	GHP 1067		0.43
GHP 1048		14.1	GHP 1068		0.15
GHP 1049		12.5	GHP 1069		0.68
GHP 1050		3.7	GHP 1070		1.6
GHP 1051		2.5	GHP 1071		0.59
GHP 1052		38.9	GHP 1072		1.05
GHP 1053		12.5	GHP 1073		0.44
GHP 1061		8.2	GHP 1074		2.64
GHP 1062		1.2	GHP 1075		0.45
GHP 1063		1.02	GHP 1076		1.10
GHP 1064		0.66	GHP 1077		1.85
GHP 1065		0.77	GHP 1078		0.43
			GHP 1079		0.48

GHP 1080		0.41	GHP 1103		2.19
GHP 1081		2.0	GHP 1104		2.8
GHP 1101		1.97	GHP 1105		0.23
GHP 1102		2.81	GHP 1106		0.35

1. A pharmaceutical composition for cancer prevention comprising an active ingredient which is sulforaphane ((-)-1-isothiocyanato-(4R)-(methylsulfinyl)butane) (CAS 4478-93-7) or an analogue thereof, said analogue having a first moiety which is an isothiocyanate and a second moiety which is a polar functional group, wherein said analogue has a chain of one or more carbon atoms linking said first and said second moieties, and wherein said analogue contains no pyridyl moieties.
2. The pharmaceutical composition of claim 1 wherein said analogue is not a heteroaromatic compound.
3. The pharmaceutical composition of claim 1 wherein said analogue is not an arylalkyl compound.
4. The pharmaceutical composition of claim 1 wherein said second moiety is a polar functional group selected from the group consisting of a carboxylic ester, a carboxylic acid, an ether, a halogen, a hydroxyl, a ketone, a nitrile, a nitro, a phosphine oxide, a sulfide, sulfone, a sulfoxide, a thioether, and a thioester.
5. The pharmaceutical composition of claim 1 wherein said second moiety is a polar functional group selected from the group consisting of a hydroxyl, a ketone, a phosphine oxide, a sulfone, and a sulfoxide.
6. The pharmaceutical composition of claim 1 wherein said chain of carbon atoms has at least three carbon atoms.

7. The pharmaceutical composition of claim 1 wherein said chain of carbon atoms has from three to five carbon atoms.
8. The pharmaceutical composition of claim 1 wherein said analogue is an olefin.
9. The pharmaceutical composition of claim 1 wherein said analogue is aliphatic.
10. The pharmaceutical composition of claim 1 wherein said chain of carbon atoms is part of a non-aromatic ring.
11. The pharmaceutical composition of claim 1 wherein said active ingredient is sulforaphane.
12. The pharmaceutical composition of claim 1 wherein said active ingredient is sulforaphene (4-isothiocyanato-(1R)-(methylsulfinyl)-1-(E)-butene) (CAS 2404-46-8).
13. The pharmaceutical composition of claim 1 wherein said active ingredient is selected from the group consisting of: 6-isothiocyanato-2-hexanon (GHP 1105); *exo*-2-acetyl-6-isothiocyanatonorbornane (GHP 1066); *exo*-2-isothiocyanato-6-methylsulfonylnorbornane (GHP 1068); 6-isothiocyanato-2-hexanol (GHP 1106); 1-isothiocyanato-4-dimethylphosphonylbutane (GHP 1078); *exo*-2-(1'-hydroxyethyl)-5-isothiocyanatonorbornane (GHP 1075); *exo*-2-acetyl-5-isothiocyanatonorbornane (GHP 1067); 1-isothiocyanato-5-methylsulfonylpentane (GHP 1003); and *cis*- or *trans*-3-(methylsulfonyl)cyclohexylmethylisothiocyanate (GHP 1079 or 1080).

14. The pharmaceutical composition of claim 1 further comprising a pharmaceutically acceptable excipient.

15. A compound which has cancer chemoprotection activity consisting of: 1-isothiocyanato-5-methylsulfonylpentane ($\text{CH}_3\text{-SO}_2\text{-(CH}_2\text{)}_5\text{-NCS}$) ((GHP 1003).

16. A compound which has cancer chemoprotection activity consisting of: 6-isothiocyanato-2-hexanone ($\text{CH}_3\text{CO(CH}_2\text{)}_4\text{NCS}$) (GHP 1105).

17. A compound which has cancer chemoprotection activity consisting of: *exo*-2-acetyl-6-isothiocyanatonorbormane (GHP 1066).

18. A compound which has cancer chemoprotection activity consisting of: *exo*-2-isothiocyanato-6-methylsulfonylnorbormane (GHP 1068).

19. A compound which has cancer chemoprotection activity consisting of: 6-isothiocyanato-2-hexanol (GHP 1106).

20. A compound which has cancer chemoprotection activity consisting of: 1-isothiocyanato-4-dimethylphosphonylbutane (GHP 1078).

21. A compound which has cancer chemoprotection activity consisting of: *exo*-2-(1'-hydroxyethyl)-5-isothiocyanatonorbormane (GHP 1075).

22. A compound which has cancer chemoprotection activity consisting of: *exo*-2-acetyl-5-isothiocyanatonorbormane (GHP 1067).

23. A compound which has cancer chemoprotection activity consisting of: *cis*- or *trans*-3-(methylsulfonyl)cyclohexylmethylisothiocyanate (GHP 1079 or 1080).

24. A method for protecting against cancer induction or progression, comprising:

administering to a mammal a chemoprotective composition consisting essentially of sulforaphane ((-)-1-isothiocyanato-(4R)-(methylsulfinyl)butane) or an analogue thereof, said analogue having a first moiety which is an isothiocyanate functionality and a second moiety which is a polar functional group, wherein said analogue has a chain of one or more carbon atoms linking said first and said second moieties, and wherein said analogue contains no pyridyl moieties, in an amount effective in producing a cancer preventive effect.

25. A food product which has been supplemented with an active chemoprotective compound, wherein said compound is sulforaphane ((-)-1-isothiocyanato-(4R)-(methylsulfinyl)butane) or an analogue thereof, said analogue having a first moiety which is an isothiocyanate functionality and a second moiety which is a polar functional group, wherein said analogue has a chain of one or more carbon atoms linking said first and said second moieties.

FIGURE 1



FIGURE 2

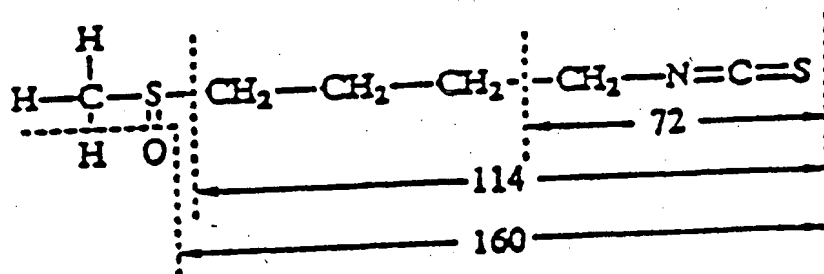


FIGURE 3

SYNTHESIS OF GHP 1003

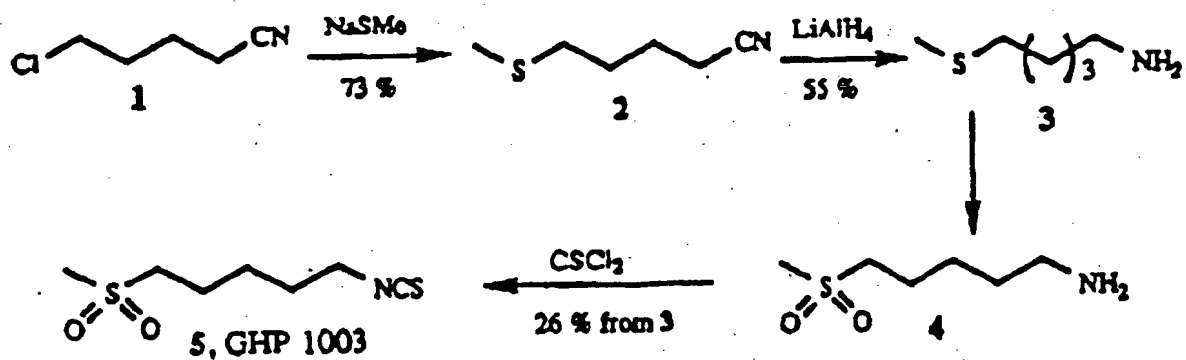


FIGURE 4

SYNTHESIS OF GHP 1066 AND GHP 1067

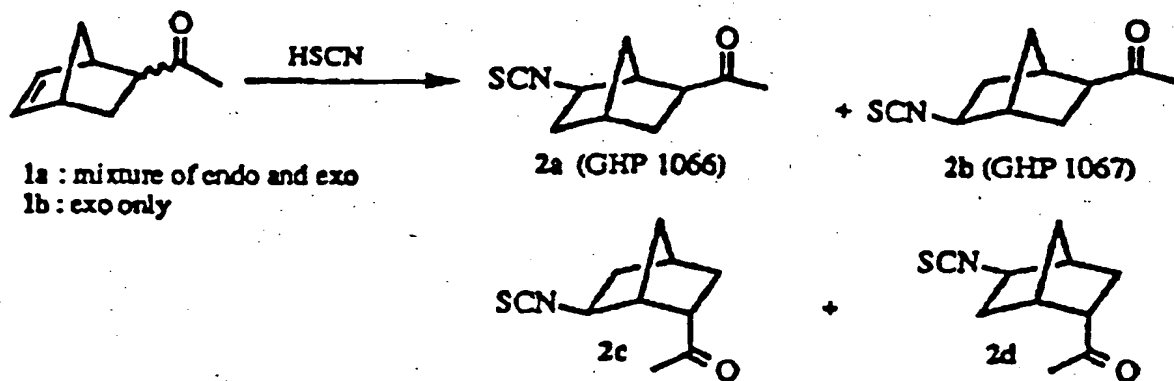


FIGURE 5

SYNTHESIS OF GHP 1064 AND GHP 1068

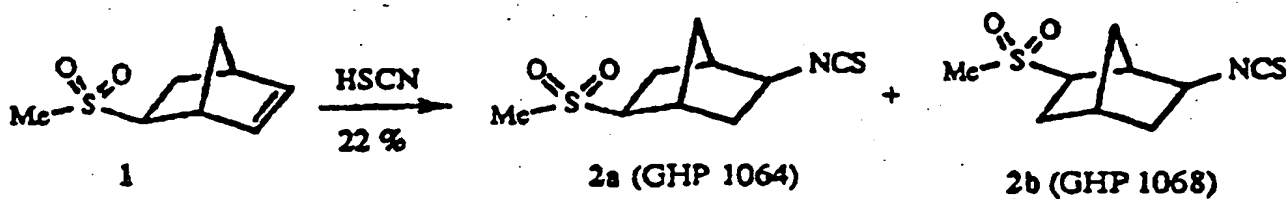


FIGURE 6

SYNTHESIS OF GHP 1073

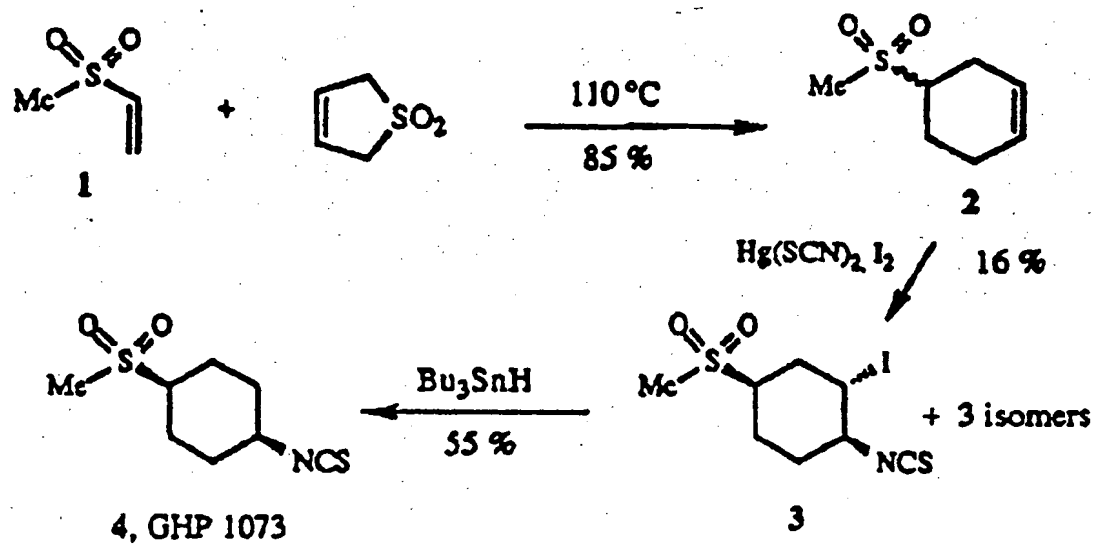


FIGURE 7

SYNTHESIS OF GHP 1075

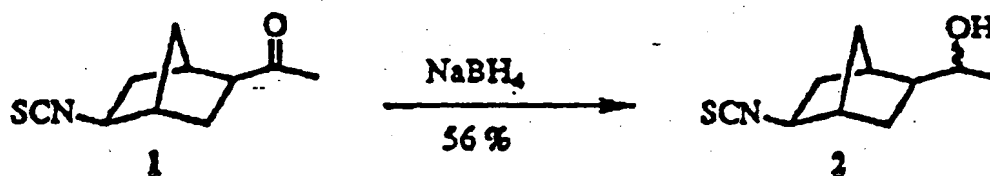


FIGURE 8

SYNTHESIS OF GHP 1078

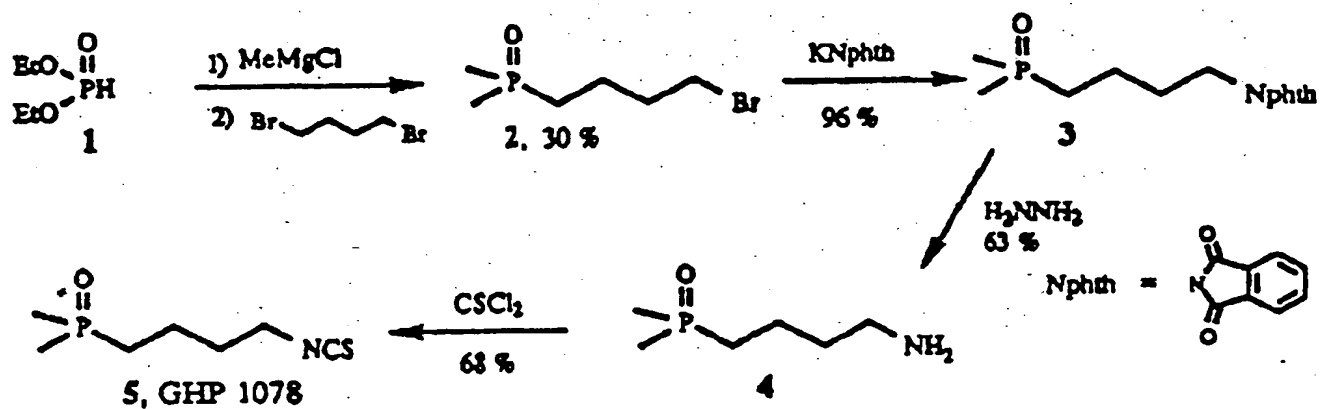


FIGURE 9

SYNTHESES OF GHP 1079 AND 1080

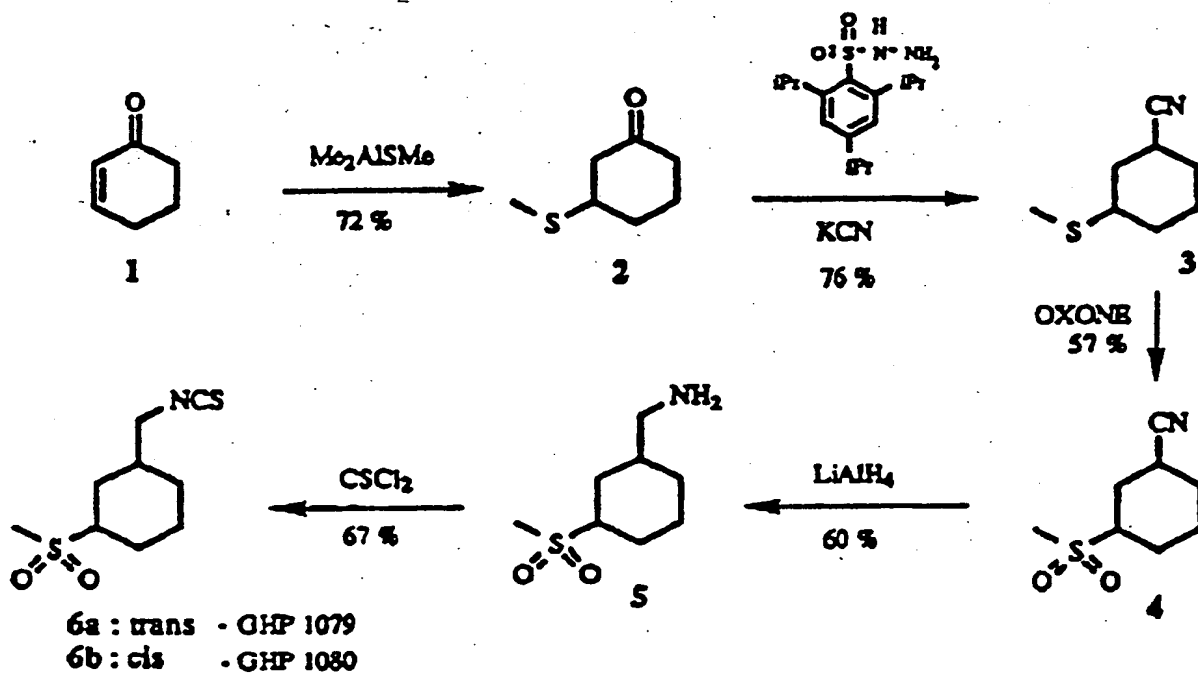


FIGURE 10

SYNTHESIS OF GHP 1105

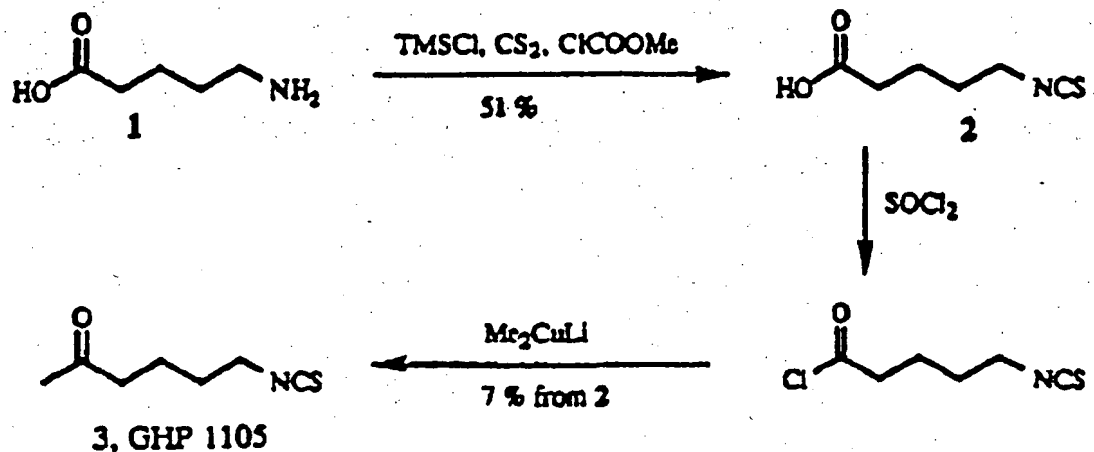
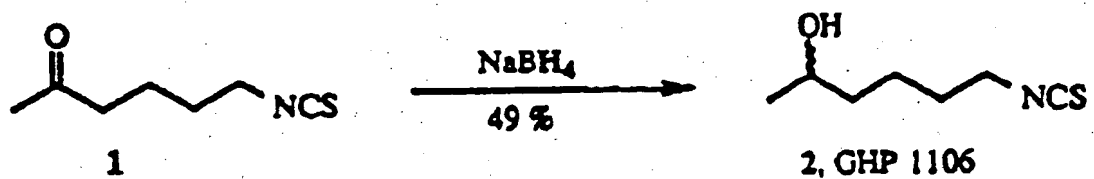


FIGURE 11

SYNTHESIS OF GHP 1106



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/02453

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A01N 47/40; C07C 331/04, 331/12

US CL : 514/514; 558/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/514; 558/17; an IPC(5): A01N 47/40; C07C 331/04, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
A	Carcinogenesis, Volume 8, No. 12, issued 1987, Lee W. Wattenberg, "Inhibitory effects of benzyl isothiocyanate administered shortly before diethylnitrosamine of benzi[a] pyrene on pulmonary and forestomach neoplasia in A/J mice", pages 1971-1973.	1-25
A	Cancer Research, Volume 51, issued 13 April 1991, Gary D. Stoner et al, "Inhibitory Effects of Phenethyl Isothiocyanate on N-Nitrosbenzylmethamphetamine Carcinogenesis in the Rat Esophagus", pages 2063-2068.	1-25

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	Z	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

17 MAY 1994

Date of mailing of the international search report

MAY 31 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

JOSEPH K. MCKANE jd

Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

